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NGS mismapping confounds~~Resolving~~ the **clinical**~~conflicting~~

interpretations of **the** *PRSSI* p.Ala16Val (c.47C>T) variant in chronic pancreatitis

**Emmanuelle Génin,^{1,2} David N. Cooper,³ Emmanuelle Masson,^{1,2} Claude Férec,^{1,2}
Jian-Min Chen,¹**

¹Inserm, Univ Brest, EFS, UMR 1078, GGB, F-29200 Brest, France

²Service de Génétique Médicale et de Biologie de la Reproduction, CHRU Brest, F-29200 Brest, France

³Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom

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Correspondence to Dr Jian-Min Chen, INSERM UMR1078 – EFS – UBO, 22 avenue Camille Desmoulins, 29238 BREST, France; Tel: +33-2-98018174; Fax: +33-2-98016474; e-mail: jian-min.chen@univ-brest.fr

We read with interest the recent publication by Weiss and colleagues, which addressed the pitfalls of using next-generation sequencing (NGS) to diagnose *PRSSI* variants in chronic pancreatitis (CP).¹ Specifically, having failed to authenticate an NGS-identified “*PRSSI* c.47C>T (p.Ala16Val) variant” by Sanger sequencing the *PRSSI* gene in the supposed carrier, they [postulated](#) that this artefact [could](#) have arisen from sequence reads emanating from one of *PRSSI*’ highly homologous and closely linked (7q34) pseudogenes, *PRSS3P2*. Herein, we address another NGS-related pitfall that contributes to confusion in relation to the clinical interpretation of *PRSSI* p.Ala16Val.

p.Ala16Val is the third most commonly detected rare [PRSSI](#) variant in [CP](#); its putative [pathological](#) involvement is supported by its ability to increase trypsinogen autoactivation.² ClinVar, [however, ascribes to](#) it conflicting interpretations of pathogenicity (i.e., likely benign (1); pathogenic (3) and uncertain significance (2)).³ The main reason for this appears to be its relatively high allele frequency (i.e., 0.006607) in all gnomAD v2.1.1 populations.⁴ Since no p.Ala16Val homozygotes were present in gnomAD, its carrier frequency would be 0.0132, which would be ~30 times the estimated prevalence of CP (i.e., 30-50/100,000).

To resolve this conundrum, we first surveyed the p.Ala16Val variant data in gnomAD v2.1.1. In all four examples of p.Ala16Val heterozygotes whose BAM files [were](#) available, a mismapping artefact mimicking gene conversion⁵ can be assumed, bearing in mind the following four considerations. First, the p.Ala16Val variant always occurs [in association](#) with another three variants in close proximity (i.e., the G-T-T-T track in [Figure 1A](#)). Second, there is a “donor” sequence for these *cis*-linked variants in another closely linked *PRSSI* pseudogene, *TRY7* (trypsinogen D; <https://www.ncbi.nlm.nih.gov/gene/?term=try7>) ([Figure 1B](#)). Third, allelic ratios of p.Ala16Val in these examples are all <25%, significantly lower

than the 50% that would be expected for a genuine heterozygous variant. Lastly, despite an [apparent](#) allele frequency of 0.006607, no p.Ala16Val homozygotes are present in gnomAD v2.1.1.[_](#) We also surveyed the p.Ala16Val variant data in the French Exome (FrEx) Project database,⁶ obtaining quite similar findings: all “p.Ala16Val” carriers (n = 159; all heterozygotes) among the 525 sequenced French individuals carry the aforementioned G-T-T-T track; [additionally](#), the allelic ratios of p.Ala16Val in these supposed heterozygous carriers are unbalanced with a maximum value of 25% ([Supplementary Figure S1](#)).

Finally, we performed a meta-analysis of studies that (i) analyzed p.Ala16Val in both patients and controls and (ii) detected the variant at least once by means of Sanger sequencing or other conventional mutation screening methods using *PRSSI*-specific primers. The meta-analysis comprised a manual survey of all *PRSSI*-related publications (until December 2018) [collated](#) in reference 2, complemented by a keyword search (*PRSSI* and “chronic pancreatitis”) in PubMed. In cases of overlapping studies from the same laboratory, the latest was used for analysis. Three eligible studies were identified;⁷⁻⁹ taken together, p.Ala16Val was [detected](#) in 18 (1.8%; all heterozygotes) of 983 CP patients but [was](#) absent from 2288 controls (odds ratio = infinity; $p < 0.001$) ([Table 1](#)).

Table 1. *PRSSI* p.Ala16Val variant in CP patients and controls derived from meta-analysis of eligible genetic studies

Study	Patients +/n	Controls +/n
Chen et al. ⁷	2/221	0/400
Rosendahl et al. ⁸	14/660	0/1758
Schubert et al. ⁹	2/102	0/130
<i>Combined</i>	18/983	0/2288

In summary, [we conclude that](#) the *bona fide* *PRSSI* p.Ala16Val variant is [\(i\)](#) extremely rare in the general population [and \(ii\) of genuine pathological significance](#). This resolves the conflicting interpretations of *PRSSI* p.Ala16Val in CP and emphasizes the need for careful use of gnomAD data in variant [assessment](#).¹⁰

Contributors EG performed the bioinformatics analysis and revised the paper. DNC, EM and CF contributed to data interpretation and revised the paper. JMC conceived the study, performed the meta-analysis and drafted the paper. All authors approved the final manuscript.

Patients and public involvement Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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Competing interests None declared

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FIGURE LEGEND

Figure 1. *PRSSI* p.Ala16Val (c.47C>T) variant as a mismapping artefact mimicking gene conversion in gnomAD v.2.1.1. (A) Partial view of genome sequencing data from a “heterozygous *PRSSI* p.Ala16Val” carrier in gnomAD v2.1.1. (B) Sequence alignment of *PRSSI* and two of its pseudogenes, *PRSS3P2* and *TRY7*. Note the presence of [a](#) “donor” sequence for the “p.Ala16Val variant and its three *cis*-linked variants” in *TRY7*.